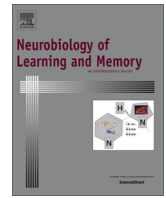




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Sleep deprivation during a specific 3-hour time window post-training impairs hippocampal synaptic plasticity and memory

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ABSTRACT

Sleep deprivation disrupts hippocampal function and plasticity. In particular, long-term memory consolidation is impaired by sleep deprivation, suggesting that a specific critical period exists following learning during which sleep is necessary. To elucidate the impact of sleep deprivation on long-term memory consolidation and synaptic plasticity, long-term memory was assessed when mice were sleep deprived following training in the hippocampus-dependent object place recognition task. We found that 3 h of sleep deprivation significantly impaired memory when deprivation began 1 h after training. In contrast, 3 h of deprivation beginning immediately post-training did not impair spatial memory. Furthermore, a 3-h sleep deprivation beginning 1 h after training impaired hippocampal long-term potentiation (LTP), whereas sleep deprivation immediately after training did not affect LTP. Together, our findings define a specific 3-h critical period, extending from 1 to 4 h after training, during which sleep deprivation impairs hippocampal function.

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1. Introduction

Despite the fact that sleep exposes an organism to increased risk of attack or predation due to time spent unaware of its surroundings, sleep is an evolutionarily conserved phenomenon that is critical for survival. Inadequate sleep contributes to both physical and mental exhaustion and deterioration. Modern society suffers from unprecedented rates of sleep loss. According to the Center for Disease Control, 7–19% of adults in the US report inadequate sleep, and an estimated 70 million Americans suffer from chronic sleep disorders. Although the vital function of sleep has yet to be determined, lack of sleep is detrimental to cognitive function.

One of the most notable negative consequences of sleep loss is impaired memory (Harrison & Horne, 2000). Memory is composed of at least three stages; acquisition, consolidation, and retrieval (Abel & Lattal, 2001). The effects of sleep deprivation have been examined on both the acquisition and consolidation of memory. Early studies explored the effects of sleep deprivation on memory acquisition (Stern, 1971), and it has since been repeatedly demonstrated that chronic sleep deprivation impairs acquisition (learning) (Abel, Havekes, Saletin, & Walker, 2013; Chee & Choo, 2004; Durmer

& Dinges, 2005; Hagewoud et al., 2010; Havekes, Vecsey, & Abel, 2012; Prince & Abel, 2013; Van Der Werf et al., 2009; Youngblood, Zhou, Smagin, Ryan, & Harris, 1997). More recently, however, multiple laboratories have explored the effects of acute sleep deprivation and sleep fragmentation during consolidation, showing that consolidation benefits from sleep and is hindered by sleep loss (Florian, Vecsey, Halassa, Haydon, & Abel, 2011; Graves, Heller, Pack, & Abel, 2003; Hagewoud et al., 2010; Hagewoud et al., 2010; Inostroza, Binder, & Born, 2013; Rolls et al., 2011; Vecsey et al., 2009).

Consolidation in hippocampus-dependent memory tasks is particularly sensitive to sleep loss. Sleep deprivation-induced deficits have been described for associative memory tasks such as contextual fear conditioning and for spatial memory tasks such as the Morris water maze task and the object-place recognition (OPR) task, which is used in the present study (Binder et al., 2012; Florian et al., 2011; Graves et al., 2003; Smith & Rose, 1996; Smith & Rose, 1997). OPR, in particular, is an ideal paradigm for examining the effects of sleep deprivation on hippocampal function because it is comparable to tasks that test declarative memory in humans, it is dependent on the hippocampus, and it is not aversive (Bussey, Duck, Muir, & Aggleton, 2000; Mumby, Gaskin, Glenn, Schramek, & Lehmann, 2002; Oliveira, Hawk, Abel, & Havekes, 2010; Shrager, Bayley, Bontempi, Hopkins, & Squire, 2007; Winters, Forwood, Cowell, Saksida, & Bussey, 2004; Winters, Saksida, & Bussey, 2008).

Several studies have assessed whether sleep deprivation during specific time windows after training affects long-term memory

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(Graves et al., 2003; Palchykova, Winsky-Sommerer, Meerlo, Dürr, & Tobler, 2006; Smith & Rose, 1997). In these studies, a sleep deprivation sensitive window, within the first 4–6 h of consolidation, has been demonstrated in the consolidation of contextual memory, object recognition memory, and spatial memory. Memory is resistant to the effects of sleep deprivation if animals are sleep deprived after this window (Graves et al., 2003; Palchykova et al., 2006; Smith & Rose, 1997). These findings suggest that there is a critical period during which memory is vulnerable to the effects of sleep deprivation. However, little work has been conducted to examine the precise timing of this sensitive time window. The aim of this study was to define the temporal parameters for the impact of sleep deprivation on memory consolidation.

Aside from the effects of sleep deprivation on behavioral measures of memory, sleep deprivation also disrupts synaptic plasticity, a neural correlate of memory. Campbell and colleagues demonstrated that 12 h of sleep deprivation impairs hippocampal long-term plasticity (LTP), a form of synaptic plasticity (Campbell, Guinan, & Horowitz, 2002). LTP deficits have been observed *in vitro* after 4–5 h of sleep deprivation as well (Kopp, Longordo, Nicholson, & Lüthi, 2006; Vecsey et al., 2009). The impact of short periods of sleep deprivation is specific to late-phase LTP (L-LTP), which requires protein synthesis and the cyclic adenosine monophosphate (cAMP) signaling pathway (Vecsey et al., 2009). Critically, however, the effects of sleep deprivation on hippocampal LTP during a period of active memory consolidation have not previously been examined. By assessing hippocampal LTP following training, in the sensitive window for sleep deprivation, we aimed to more accurately determine the effects of sleep and sleep loss on hippocampal plasticity associated with memory consolidation.

Previously, we demonstrated that as little as 6 h of sleep deprivation immediately after task training disrupts long-term spatial memory in OPR (Florian et al., 2011). Here we aim to better define the critical period during which sleep is essential for hippocampal memory consolidation. By sleep depriving mice during two different time windows, we demonstrate that as few as 3 h of sleep deprivation during consolidation can affect both long-term memory and LTP.

2. Methods

2.1. Subjects

One hundred C57BL/6J adult male mice (2–4 months of age) were pair housed and kept on a 12 h/12 h light/dark schedule with lights on at 7:00 AM (ZT 0). Food and water were available *ad*

libitum throughout the experiments. All experiments were approved by the Institution of Animal Care and Use Committee of the University of Pennsylvania and were carried out in accordance with all National Institutes of Health guidelines.

2.2. Sleep deprivation

To assess the effects of sleep deprivation (SD) on memory, mice ($n = 58$) were sleep-deprived using the gentle handling technique involving manual cage tapping, cage shaking, nestlet disturbance, and gentle animal prodding (Ledoux, Sastre, Buda, Luppi, & Jouvet, 1996; Vecsey et al., 2013). Prior work using electroencephalographic recordings has shown that this procedure effectively retains animals in a state of wakefulness for several hours (Meerlo, De Bruin, Strijkstra, & Daan, 2001). The frequency of these manipulations was monitored throughout the sleep deprivation period (Fig. 5A and B). Separate groups of mice were sleep deprived in one of the two 3-h periods for behavior and electrophysiology experiments (ZT 1–4 and ZT 2–5) after behavioral training as described in Fig. 1A and B. Non-sleep deprivation (NSD) time-matched control groups were used for comparison with the two SD experimental groups.

2.3. Object-place recognition (OPR)

For this task, we used a previously established design that has been shown to be hippocampus dependent (Fig. 1A; Havekes, Canton, et al., 2012; Havekes, Vecsey, et al., 2012; Oliveira et al., 2010). Mice ($n = 80$) were handled for 2 min each day, for 6 consecutive days leading up to experimentation. The task was conducted in a grey rectangular box (40 cm × 30 cm × 30 cm) built of polyvinyl chloride plastic. At the beginning of the light phase (ZT 0), mice were placed in the empty box for 6 min for habituation. Mice were then removed and placed back in the home cage. After 3 min, mice were placed in the box with 3 different objects (a 100 ml glass bottle, a white cylinder, and a metallic rectangular tower) for 3 consecutive 6-min training sessions. Each training session was separated by a 3-min interval during which the animals were returned to the holding cages. At completion of the training sessions, NSD mice were left undisturbed in their home cages and SD mice were deprived of sleep by gentle handling. Twenty-four hours following the training session, mice were re-introduced to the spatial context in a single test session. In this session, one of the objects was repositioned (the displaced object: DO), thereby changing the spatial configuration of the objects in the box. Mice were allowed to explore objects for 6 min. Exploration was recorded during training

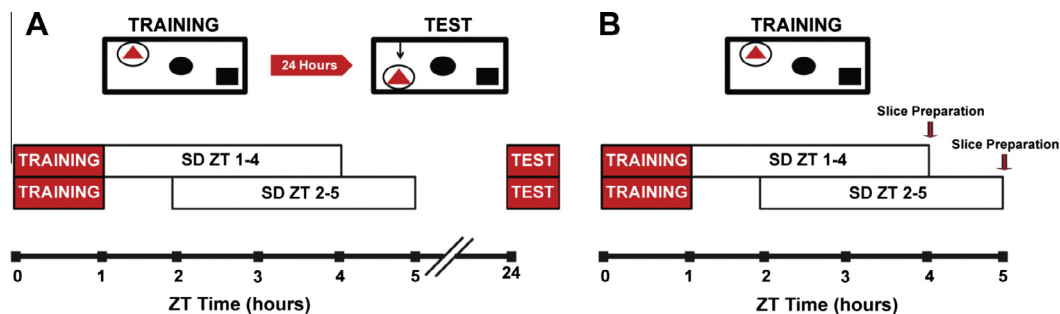


Fig. 1. Schematic depicting the behavioral and LTP experimental design. (A) Behavioral experimental design: The top diagram depicts the OPR task used to examine hippocampus-dependent memory. The 3 training sessions include repeated exposure to 3 distinct objects. Training sessions began at lights on ZT 0. The test session occurred 24 h following training. The bottom diagram depicts the post-training sleep deprivation time periods for each behavioral experiment. After the last training session, mice were subjected to either an immediate or delayed 3-h sleep deprivation period to assess the specific time window for sleep deprivation to impair memory. (B) LTP experimental design: The top diagram demonstrates that mice were subjected to the same training as those that were in the behavioral experiment assessing memory. However, there was no later test period for these animals. The bottom diagram depicts the sleep deprivation periods after training as well as when hippocampal slices were collected for field recordings. Recordings obtained from NSD control groups were later pooled, and LTP from the sleep deprivation groups were compared to this pooled group. Prior to pooling the data from the NSD control groups, we determined that the NSD control groups were not significantly different from each other.

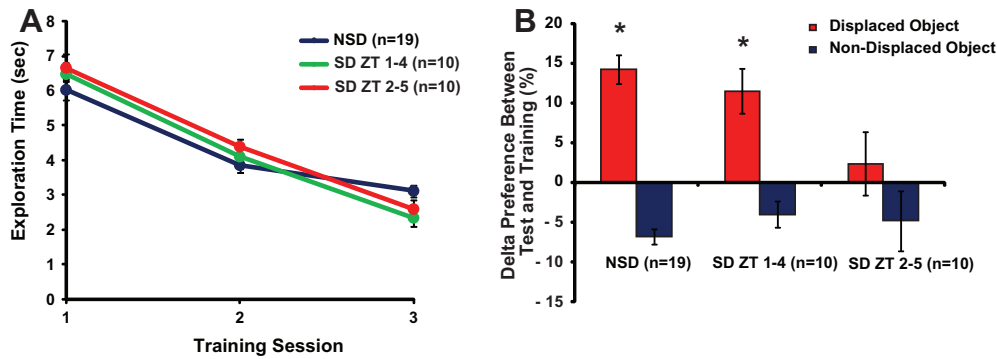


Fig. 2. A specific 3-h sleep deprivation period impairs spatial memory. (A) Mean exploration time of objects during each session was not significantly different between the 3 experimental groups ($F_{(4,72)} = 1.85, p = 0.13$). Mean exploration time gradually decreased during training ($F_{(2,72)} = 199.88, p < 0.05$) (blue = NSD, green = SD ZT 1–4, red = SD ZT 2–5). (B) Mean delta percentage of exploration of NDO and DO between test session and final training session for SD and NSD groups. Mean delta percentage of DO exploration significantly increased for the NSD group ($n = 19$) compared to their exploration of the NDO ($F_{(2,36)} = 3.24, p < 0.05; t_{(36)} = 6.75, p < 0.05$). Mean delta percentage of DO exploration significantly increased for SD ZT 1–4 group ($n = 10$) compared to their exploration of the NDO ($F_{(2,36)} = 3.24, p < 0.05; t_{(36)} = 3.66, p < 0.05$). For mice subjected to a delayed SD from ZT 2–5 ($n = 10$), mean delta percentage of DO exploration was not significantly different from percentage exploration of the NDO ($F_{(2,36)} = 3.24, p < 0.05; t_{(36)} = 1.70, p = 0.53$). Error bars indicate \pm SEM. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

and testing on a digital camera for subsequent scoring of time spent exploring objects. Exploration of the objects was defined as the amount of time mice were oriented toward an object with its nose within 1 cm of it, and was scored by an experimenter blind to sleep group.

The change in preference of object exploration between the last training session and test session was used as an index for spatial memory. Preference for an object during the training and test session was determined by calculating the ratio of time, in seconds, spent at a particular object versus total time spent at all objects. The equation for this measure is as follows: $[\text{TEST} (\text{DO}/(\text{DO} + \text{NDO}) \times 100) - 3\text{rd TRAINING SESSION} (\text{DO}/(\text{DO} + \text{NDO}) \times 100)]$, where DO = time spent exploring displaced object (prior to relocation and once relocated) and NDO = time spent exploring the non-displaced objects. A positive change in percentage or exploration time between test session and the final training session of displaced object indicates a preference for the displaced object. This is indicative of an accurate memory for the original locations of objects and that the mice were able to recognize the object's displacement. No change or a negative change in time spent exploring the displaced object in the test session compared to the last training session indicates inability to recognize the new spatial configuration.

2.4. Electrophysiology

To assess the effects of SD after OPR training on hippocampal LTP, electrophysiological recordings were performed as described previously (Vecsey et al., 2009). Animals were trained in the OPR paradigm at ZT 0 ($n = 19$). They were then either left undisturbed in their home cages (NSD control group, $n = 9$), or subjected to 3 h of SD. Animals were sleep deprived for 3 h either directly after training (OPR + SD ZT 1–4 group, $n = 5$) or 1 h after training (OPR + SD ZT 2–5 group $n = 5$) (Fig. 1B). A separate group of naïve mice that did not receive OPR training were either sleep deprived from ZT 2–5 (SD ZT 2–5 group, $n = 6$) or left undisturbed in their home cages (NSD group, $n = 8$). Immediately after sleep deprivation, SD and time-matched NSD mice were sacrificed by cervical dislocation, and their hippocampi were dissected rapidly in iced oxygenated artificial cerebrospinal fluid (aCSF; 124 mM NaCl, 4.4 mM KCl, 1.3 mM $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 1 $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$, 26.2 mM NaHCO_3 , 2.5 mM $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 10 mM glucose). 400 μm thick transverse hippocampal slices were prepared using a Stoelting tissue chopper (Stoelting Co. Wood Dale, IL) and placed in an interface chamber and continuously perfused with oxygenated aCSF while they equilibrated for at least 1.5 h at 28.0 °C before starting

electrophysiological recordings. Single pathway recordings were made using a single bipolar stimulating electrode (A-M Systems; 0.002 inches diameter nichrome wire) placed in the stratum radiatum of the CA1 subfield to elicit action potentials in the axons of CA3 pyramidal neurons. An aCSF-filled glass microelectrode (A-M Systems; 1.5 mm \times 0.85 mm) with a resistance between 0.5 and 3 M Ω placed in the stratum radiatum region of CA1 was used to record the resulting field excitatory post-synaptic potential (fEPSP). Data were acquired and analyzed using Clampex 8.2 software (Molecular Devices, Palo Alto, CA). Peak fEPSPs amplitude was required to be at least 5 mV, and stimulus intensity was set to produce 40% of the maximal response. Test stimulation occurred once every minute. A 20-min baseline period was recorded in each experiment prior to stimulation. To examine late-phase LTP (L-LTP), 4 trains of stimuli at 100 Hz for 1 s were delivered 5 min apart, otherwise known as spaced 4-train high frequency stimulation. Recordings continued for at least 2 h after LTP induction. Initial fEPSP slopes were normalized against the average of the 20 baseline traces. Input–output characteristics in area CA1 were investigated by recording the fEPSPs elicited by stimuli of increasing intensity after maximum fEPSP was determined. The initial fEPSP slopes were plotted against the amplitudes of corresponding presynaptic fiber volleys and fit with linear regressions. The maximum elicited fEPSP slope was also recorded as a measure of synaptic strength. Paired-pulse facilitation, a short-term form of synaptic plasticity and a measure of presynaptic function, was measured in slices from NSD and SD groups. Paired stimuli were delivered with varying interpulse intervals (300, 200, 100, 50, and 25 ms) and the fEPSP slope from the 2nd stimulus was plotted relative to the slope from the 1st stimulus to give the facilitation ratio. We combined NSD control data from the 2 experimental groups as they were not significantly different from each other.

2.5. Polysomnography

To determine if undisturbed sleep during these 2 time periods differed, sleep was measured using polysomnography. Naïve mice ($n = 6$) had electrodes implanted to record EEG and EMG signals and assess time spent in wakefulness, NREM, and REM sleep.

Adult male C57BL/6 J mice were implanted with electroencephalographic (EEG) and electromyographic (EMG) electrodes under isoflurane anesthesia. EEG electrodes were implanted 1.5 mm bilateral to midline and 1.5 mm posterior to bregma on each side using an electric drill. A reference EEG electrode was placed 1.5 mm posterior to lambda over the cerebellum (Hellman,

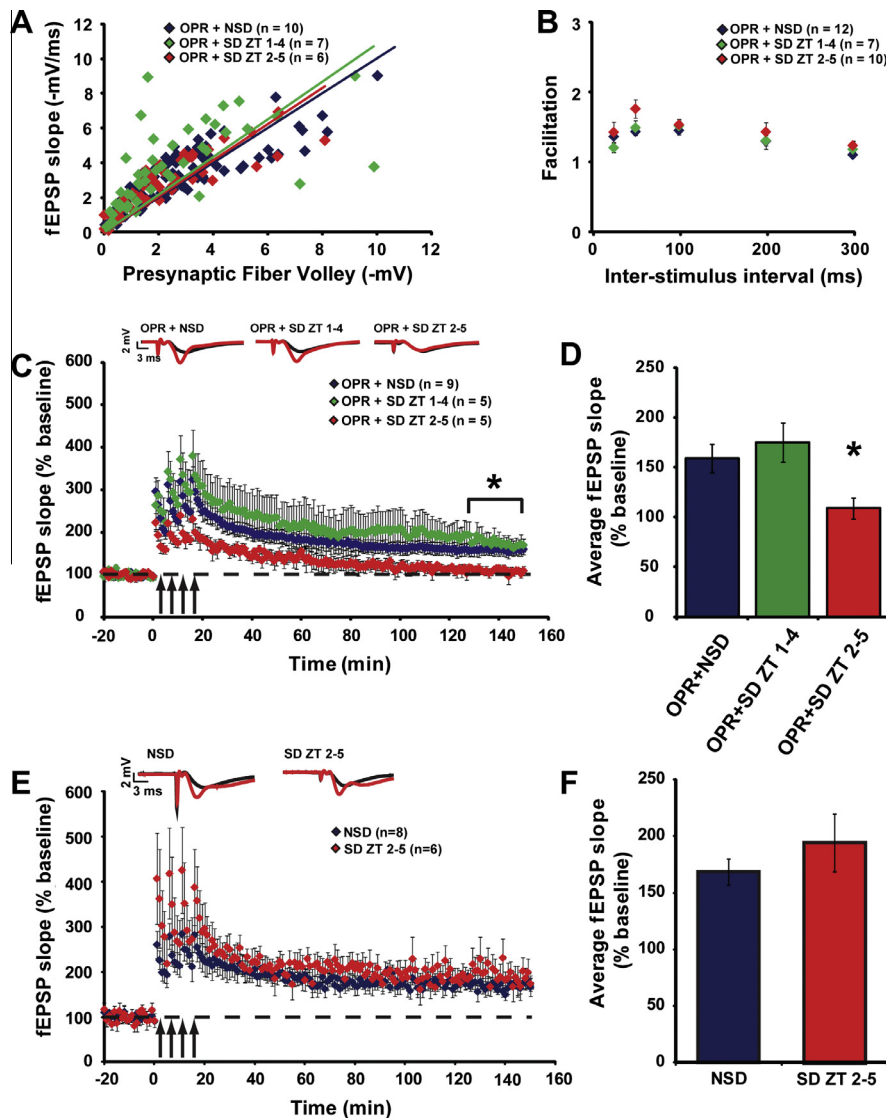


Fig. 3. Delayed sleep deprivation after object-place recognition training disrupts LTP. (A) Input–output curves relating the amplitude of the presynaptic fiber volley to the initial slope of the corresponding fEPSP at various stimulus intensities were not altered in slices from either OPR trained SD groups compared to the OPR trained NSD group ($F_{(2,20)} = 2.96$, $p = 0.08$). (B) Paired-pulse facilitation, a short-term form of synaptic plasticity, did not differ in slices obtained from either of the OPR trained SD groups compared to slices from the OPR trained NSD group ($F_{(8,100)} = 1.38$, $p = 0.21$). (C) L-LTP induced by spaced 4-train stimulation was not impaired in slices from OPR trained animals in the NSD group (blue, $n = 9$). L-LTP induced by spaced 4-train stimulation was not impaired in slices from the OPR + SD ZT 1–4 group (green, $n = 5$). L-LTP induced by spaced 4-train stimulation was attenuated in slices from the OPR + SD ZT 2–5 group (red, $n = 5$) compared to the OPR + NSD group. (D) Bar graph depicts the average fEPSP slope over the final 20 min of recording for OPR + NSD, OPR + SD ZT 1–4, and OPR + SD ZT 2–5 group ($F_{(2,16)} = 4.85$, $p < 0.05$). *Post hoc* tests showed that the SD ZT 2–5 group was significantly different from both NSD and SD 1–4 group ($p < 0.05$). (E) L-LTP induced by spaced 4-train stimulation was not impaired in slices from *non-trained* SD ZT 2–5 group compared (red, $n = 6$) to *non-trained* NSD control group (blue, $n = 8$). (F) Bar graph depicts the average fEPSP slope over the final 20 min of the recording for NSD and SD ZT 2–5 group ($t_{(12)} = -1.09$, $p = 0.30$). In all sample sweeps, black traces indicate baseline, and red traces were acquired ~2 h after stimulation. Calibration: 2 mV, 3 ms. Error bars indicate SEM. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Hernandez, Park, & Abel, 2010; Huber, Deboer, & Tobler, 2000; Vecsey et al., 2013). EMG electrodes were attached to nuchal musculature. Electrodes were held in place with glass ionomer resin (Ketacem, 3M, Maplewood, MN, Chemelli et al., 1999). Electrodes consisted of Teflon coated wires (Cooner wires, Chatsworth, CA) soldered to gold socket contacts (Plastics One, Roanoke, VA) and pushed into a 6-pin plastic plug (363 plug, Plastics One), which was then attached to a commutator (SLC6, Plastics One; (Vecsey et al., 2000)). All recordings were analyzed with the right EEG electrode and were referenced to the cerebellum electrode. This electrode-referencing scheme is ideal for detecting alterations in delta and theta activity in mice (Franken, Lopez-Molina, Marcacci, Schibler, & Tafti, 2000; Tafti et al., 2003). Mice were housed individually and allowed 2 weeks of recovery after surgery. During the second week of recovery, mice were acclimated to the

recording chambers and to the cables. During the second week, mice were handled daily for 2 min each (Vecsey et al., 2013).

EEG/EMG signals were sampled at 256 Hz and filtered at 0.5–30 Hz and 1–100 Hz, respectively, with 12A5 amplifiers (Astro-Med, West Warwick, RI). Data acquisition and visual scoring was performed using SleepSign software (Kissei Comtec, Inc. Japan). EEG/EMG recordings were scored in 4-s epochs as wake, non-rapid eye movement (NREM) sleep, or rapid eye movement (REM) sleep by a trained experimenter blind to experimental conditions.

2.6. Statistical analysis

Two-way ANOVA was used to analyze spatial preference in the OPR task, with sleep group and objects as factors and the delta

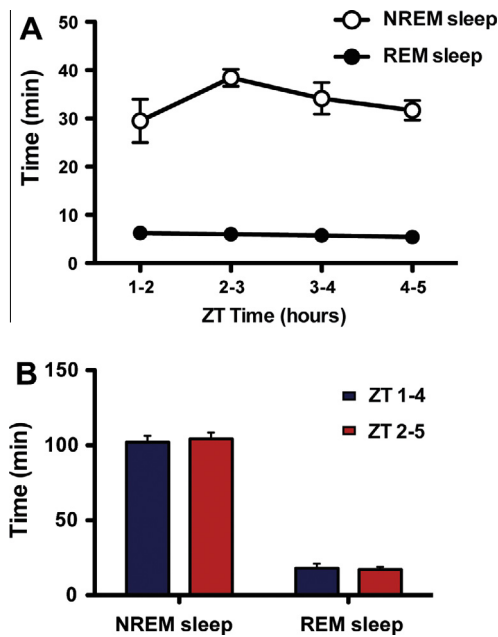


Fig. 4. Mice exhibit similar sleep patterns during periods ZT 1–5. (A) Mean time in minutes spent in NREM and REM sleep during each hour across ZT 1–5 is shown. Mice ($n = 6$) spend comparable time in NREM sleep for each hour displayed. Mice also spend comparable time in REM sleep for each hour displayed ($F_{(3,20)} = 1.090$; $p = 0.3763$). (B) Mean time in minutes spent in NREM sleep and REM sleep from ZT 1–4 and ZT 2–5 is shown. Mice ($n = 6$) spend comparable time in NREM sleep as well as REM sleep during these periods. Error bars indicate \pm SEM.

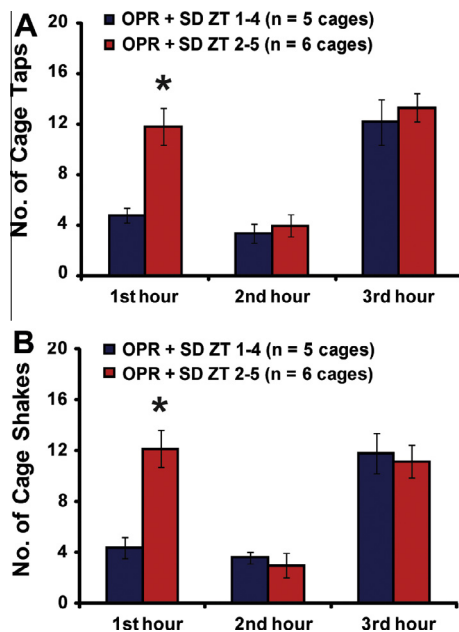


Fig. 5. The early sleep deprivation group requires less disturbance to achieve a wakeful state in the 1st hour of SD than the SD ZT 2–5 group. (A) Mean number of cage taps required to maintain wakefulness across 3 h of SD after object-place recognition training. Frequency of cage taps in the 1st hour of sleep deprivation was significantly higher in the SD ZT 2–5 group ($n = 6$) than the SD ZT 1–4 group ($n = 5$) ($F_{(2,18)} = 5.67$, $p < 0.05$; $t_{(18)} = 4.60$, $p < 0.05$). (B) Mean number of cage shakes required to maintain wakefulness across 3 h of SD after object-place recognition training. Frequency of cage shakes in the 1st hour of SD was significantly higher in SD ZT 2–5 group than the SD ZT 1–4 group ($F_{(2,18)} = 10.19$, $p < 0.05$; $t_{(18)} = 4.82$, $p < 0.05$). Error bars indicate \pm SEM.

percent exploration as the dependent variable. One-way ANOVAs were used to analyze input output curves and to analyze the average of the last 20 min of fEPSP slopes from potentiated

hippocampal slices from the OPR + NSD group and the 2 OPR + SD groups. A student's t-test was used to analyze the average of the last 20 min of fEPSP slopes from potentiated hippocampal slices from *non-trained* NSD and SD ZT 2–5 groups. Two-way repeated measures ANOVAs were used to analyze paired pulse facilitation, time spent in NREM and REM sleep, and gentle handling during SD. Tukey's multiple comparison was selected for *post hoc* analyses for OPR, gentle handling. Dunnett's multiple comparison was selected for *post hoc* analyses of hippocampal synaptic plasticity. All statistical analyses were performed using SAS 9.3 software (SAS Institute Inc., Cary, NC). A probability level of $p < 0.05$ was considered significant. Data are presented as mean \pm SEM.

3. Results

3.1. Delayed sleep deprivation during hours ZT 2–5 after object place recognition (OPR) training impairs long-term memory

We first examined the impact of two periods of SD during the consolidation of spatial memory. We subjected separate groups of mice to one of two overlapping 3-h SD periods during the first 4 h of consolidation. We chose to examine these overlapping sleep deprivation time points because we had already discovered that 4 h of immediate sleep deprivation (ZT 1–5) was sufficient to impair memory compared to the NSD control group. This observation suggests that the critical time window necessary for sleep exists within the first 4 h of after learning in mice. A two way ANOVA revealed an interaction effect between change in exploration of the displaced object [DO] and sleep group ($F_{(1,14)} = 13.07$, $p < 0.05$; NSD DO = $19.02 \pm 4.34\%$, SD DO = $-0.47 \pm 4.00\%$; $p < 0.05$) ($F_{(1,14)} = 13.07$, $p < 0.05$; SD NDO = $1.03 \pm 2.16\%$, SD DO = $-0.47 \pm 4.00\%$; $p = 0.99$). Mice were sleep deprived for 3 h immediately after training (ZT 1–4; immediate SD) or for 3 h beginning an hour after training (ZT 2–5; delayed SD), as outlined in Fig. 1A. The three groups (NSD, immediate SD, and delayed SD) acquired the task at similar rates, measured by time spent exploring objects during each session (Fig. 2A). A two-way repeated measures ANOVA revealed that the three groups did not differ in exploration times ($F_{(2,36)} = 0.32$, $p = 0.73$). All groups gradually reduced total exploration time ($F_{(2,72)} = 199.88$, $p < 0.05$). No interaction existed for session and group for exploration time ($F_{(4,72)} = 1.85$, $p = 0.13$). Two-way ANOVA revealed that SD group had an effect on the extent of change in preference for the objects between the 3rd training session and the test session (delta preference) ($F_{(2,36)} = 3.88$, $p < 0.05$). There was a significant main effect of type of object (non-displaced object [NDO] vs. displaced object [DO]) in delta preference of object exploration ($F_{(1,36)} = 41.59$, $p < 0.05$). There was a significant interaction between sleep group and type of object ($F_{(2,36)} = 3.24$, $p < 0.05$). *Post hoc* analysis revealed that the NSD group exhibited enhanced preference for the DO during the test session relative to the NDO (NDO = $-6.54 \pm 1.24\%$, DO = $14.2 \pm 1.99\%$; $p < 0.05$). The immediate SD group exhibited increased exploration of the DO compared to the NDO (NDO = $-4.0 \pm 3.78\%$, DO = $11.52 \pm 3.96\%$; $p < 0.05$). There was no difference in exploration of the NDO between the NSD group and the immediate SD group (NSD = $-6.54 \pm 1.13\%$, SD ZT 1–4 = $-4.0 \pm 3.78\%$; $p = 0.97$). Similarly, there was no difference in the preference for the DO in the immediate SD group compared to the control NSD group (NSD = $14.2 \pm 1.99\%$, SD ZT 1–4 = $11.52 \pm 3.96\%$; $p = 0.96$) (Fig. 2B). Analysis of the delayed SD group (Fig. 2B) revealed that preference for the DO was not significantly different compared to the NDO (NDO = $-4.82 \pm 1.63\%$; DO = $2.38 \pm 2.82\%$; $p = 0.54$). There was significantly decreased exploration of the DO in the delayed SD group compared to the NSD group (NSD = $14.2 \pm 1.99\%$; SD ZT 2–5 = $2.38 \pm 2.82\%$; $p < 0.05$). *Post hoc* analysis revealed that the exploration of the

NDO was not significantly different in the delayed SD group compared to NSD controls (NSD = $-6.54 \pm 1.24\%$; SD ZT 2–5 = $-4.82 \pm 1.63\%$; $p = 0.99$). Thus, our data indicate that there is a specific time window, during ZT 2–5, where object-place memory consolidation is vulnerable to sleep deprivation.

3.2. Delayed sleep deprivation during hours ZT 2–5 after object-place recognition training disrupts LTP

Due to the distinct behavioral outcomes that resulted from the immediate and delayed 3 h periods of sleep deprivation, we examined hippocampal synaptic function and L-LTP in area CA1 after OPR training followed by these 2 time periods of sleep deprivation. Input output curves did not differ between NSD, immediate SD, and delayed SD groups (Fig. 3B; $F_{(2,20)} = 2.96$, $p = 0.08$). Paired pulse facilitation (PPF) was not altered in either of the SD groups compared to the NSD group after training (Fig. 3A; $F_{(8,100)} = 1.38$, $p = 0.21$). Synaptic strength, measured as the maximum fEPSP slope induced by stimulation, was comparable between groups (OPR + NSD = 7.2 ± 0.42 -mV/ms; OPR + SD ZT 1–4 = 7.4 ± 0.60 -mV/ms; OPR + SD ZT 2–5 = 7.0 ± 0.39 -mV/ms) ($F_{(2,36)} = 0.13$, $p = 0.88$). We compared L-LTP from both the delayed and immediate SD groups to a NSD control group. A one-way ANOVA on the average of the last 20 min of recordings in each group revealed that sleep group had an effect on L-LTP, $F_{(2,16)} = 4.85$, $p < 0.05$. *Post hoc* analysis revealed that L-LTP was elicited in slices from the immediate SD group, the potentiation was not significantly different compared to slices from NSD control group (Fig. 3C and Fig. 3D; OPR + NSD = $159\% \pm 14.33\%$, OPR + SD ZT 1–4 = $175\% \pm 19.67\%$; $p = 0.71$). Conversely, the delayed SD group displayed impaired LTP compared to the NSD control group and the immediate SD group (OPR + SD ZT 2–5 = $109\% \pm 10.50\%$; $p < 0.05$). We also examined the effect of sleep deprivation on LTP during the ZT 2–5 time window without prior behavioral training. A student's *t*-test on the averaged fEPSP slope of the last 20 min of recordings in the SD ZT 2–5 group compared to the NSD group revealed that 3 h of sleep deprivation without prior training did not affect LTP ($t_{(12)} = -1.09$, $p = 0.30$) (Fig. 3E and F). This pinpoints a specific 3-h time window after learning where sleep deprivation during consolidation disrupts not only memory, but hippocampal LTP as well.

3.3. Mice spent similar time in NREM and REM sleep throughout the period ZT 1–5

Following these results, we wanted to examine whether sleep differed during these two periods and thereby, could potentially account for the difference in the effect of sleep deprivation on memory and plasticity. We measured NREM and REM sleep in mice from ZT 1–5 using polysomnography. We compared the time spent in NREM and REM each hour from ZT 1 to ZT 5 (NREM sleep in minutes: ZT 1–2 = 29.49 ± 4.49 , ZT 2–3 = 38.44 ± 1.74 , ZT 3–4 = 34.16 ± 3.26 , ZT 4–5 = 31.69 ± 2.06 ; REM sleep in minutes: ZT 1–2 = 6.26 ± 1.45 , ZT 2–3 = 6.03 ± 1.12 , ZT 3–4 = 5.76 ± 0.78 , ZT 4–5 = 5.43 ± 0.61). We found no difference in time spent in NREM or REM sleep from ZT1 to ZT 5 (Fig. 4; $F_{(3,20)} = 1.090$; $p = 0.3763$).

3.4. Increased gentle handling was required to maintain wakefulness in the delayed sleep deprivation group in comparison to the immediate sleep deprivation group

To determine if the amount of stimulation needed to keep animals awake differed between the two SD periods, the frequency of manipulation during each hour of sleep deprivation was analyzed. For cage taps (Fig. 5A), there was a significant treatment group by hour interaction ($F_{(2,18)} = 5.67$, $p < 0.05$). *Post hoc* analyses indicated that the number of cage taps to maintain the wake state

was significantly greater in the first hour for the delayed group compared to the first hour in the immediate SD group (OPR + SD ZT 1–4 = 4.8 ± 0.55 , OPR + SD ZT 2–5 = 11.83 ± 1.48 ; $p < 0.05$). The frequency of cage shaking was also measured across the two 3-h SD groups (Fig. 5B). A two-way repeated measures ANOVA revealed an interaction between hour and treatment group ($F_{(2,18)} = 10.19$, $p < 0.05$). *Post hoc* analyses revealed that compared to the immediate SD group, animals from delayed SD group required more cage shakes to sustain wakefulness during the first hour of the 3-h SD (OPR + SD ZT 1–4 = 4.4 ± 0.84 , OPR + SD ZT 2–5 = 12.17 ± 1.48 ; $p < 0.05$). Our findings indicate that the immediate SD group required less gentle handling manipulation to maintain wake than the delayed sleep deprivation group within the 1st hour.

4. Discussion

Our study investigated whether there is a critical time window for sleep deprivation during memory consolidation to impair hippocampus-dependent long-term memory and plasticity. We found that as little as 3 h of SD following learning impairs spatial memory in mice. More importantly, we observed that the timing of sleep loss is critical. When the onset of SD was delayed for an hour after learning, hippocampus-dependent memory consolidation was impaired compared to when mice were sleep deprived beginning immediately after learning (Fig. 2B). We also found, to the best of our knowledge, the first evidence that SD after learning impaired hippocampal plasticity, but only when delayed by one hour following training (Fig. 3D). Thus memory consolidation and synaptic plasticity exhibit a similar sensitivity to sleep deprivation.

After determining that the delayed 3-h SD period is sufficient for the manifestation of memory and plasticity impairments, we examined potential factors that could explain these 2 different 3-h periods of sleep deprivation produced distinct outcomes. First, it was possible that there are qualitative differences in the type of sleep engaged in between these two periods. Previous findings have associated periods of more NREM sleep with stronger consolidation of hippocampus-dependent memories (Plihal & Born, 1997; van der Helm, Gujar, Nishida, & Walker, 2011; Wamsley, Tucker, Payne, & Stickgold, 2010). If animals were sleep deprived during a time characterized by more NREM sleep, this could possibly explain our findings. We found that mice exhibited similar NREM and REM sleep across the hours ZT 1–5 and during the ZT 1–4 and ZT 2–5 periods (Fig. 4A and B). This suggests that other factors must be at work in producing our main finding.

On the other hand, although naïve animals did not show differences in sleep patterns throughout ZT 1–5 (Fig. 4A), modulation in sleep due to prior training could affect the amount of sleep lost in the delayed SD condition compared to the sleep lost in immediate SD condition. Previous studies have indicated that training in open field, novelty exposure, as well as handling leads to an immediate decrease in sleep followed by increases in later periods of sleep (Hellman & Abel, 2007; Tang, Liu, Yang, & Sanford, 2005; Tang, Xiao, Liu, & Sanford, 2004; Tang, Yang, & Sanford, 2007). Other studies, however, showed an increase in REM sleep following learning in the water maze task and inhibitory avoidance task (Smith, 1985; Smith & Rose, 1996). The present study supports these findings of altered sleep patterns after training. Our assessment through measurement of gentle handling showed that the immediate SD group required less manipulation to maintain wake than the delayed sleep deprivation group within the first hour, lending further support to previous findings that training changes sleep patterns in animals (Fig. 5A and B). The delayed SD group required more manipulation throughout the SD procedure, which could be a result of increased sleep pressure in the delayed group. We think that memory deficits observed in the delayed SD group

could be attributed to a combination of sleep deprivation and sleep pressure rather than the increased frequency of stimulation during SD. In line with our findings, a previous study from Hagewoud and colleagues (2010) also showed that impaired memory was a result of extent of sleep loss and not due to frequency of stimulation needed to keep animals awake (Hagewoud, Whitcomb, et al., 2010). Furthermore, our work suggests that delayed SD is potentially more disruptive than immediate SD due to prior training affecting later sleep, therefore enhancing the impact of sleep deprivation during the later time period. This may explain why the delayed SD without prior behavioral training had no effect on LTP (Fig. 3E and F), while prior training followed by delayed SD impaired LTP (Fig. 3C and D). More extensive examination is needed in future, including recording sleep after OPR learning and measuring slow wave activity over the hours following OPR training. Other groups have used various instrumentation techniques to record from the cortex, as well as the hippocampus, in experiments that involved another object recognition task, novel object recognition, so this may be a future area of interest (Clarke, Cammarota, Gruart, Izquierdo, & Delgado-García, 2010; Rolls et al., 2011).

Another potential reason that delayed SD would impair the consolidation of object-place memory is that this delayed period of SD may disrupt molecular signaling pathways known to be important for memory consolidation. For instance, SD has previously been shown to reduce levels of the second messenger, cAMP in the hippocampus (Vecsey et al., 2009). cAMP signaling in the hippocampus has been observed to peak at 3 h after acquisition, along with α -amino-3-hydroxy-5-methyl-isoxazolepropionate (AMPA) receptor and dopamine (D1) receptor function (Bernabeu, Cammarota, Izquierdo, & Medina, 1997; Bernabeu et al., 1997). Also, cAMP signaling in the hippocampus has been observed to oscillate in a circadian fashion with increases from ZT 4 through ZT 8 (Eckel-Mahan et al., 2008). Luo and Colleagues showed that this period of increased cAMP levels occurred during REM sleep, accompanied by increases in mitogen activated protein kinase (MAPK) and cAMP-response element binding protein (CREB) levels (Luo, Phan, Yang, Garelick, & Storm, 2013). Elevated CREB expression required for consolidation occurs both immediately after and 4 h after acquisition (Bourtchouladze et al., 1998). Because our delayed SD manipulation occurs during the period of increased signaling necessary for memory consolidation, it is likely preventing consolidation by disrupting these active signaling cascades. Thus, the memory and hippocampal plasticity deficits that were observed in the delayed SD group could be attributed to disrupted activation of the molecular signaling pathways necessary for memory consolidation, which selectively occurs in the later time period. Future experiments will be needed to explore these ideas.

Impairment in the maintenance of hippocampal LTP due to total sleep deprivation and REM sleep deprivation has been observed in freely moving animals (Ishikawa et al., 2006; Romcy-Pereira & Pavlides, 2004) and in hippocampal slices prepared from sleep-deprived animals (Campbell et al., 2002; Kopp et al., 2006; McDermott et al., 2003; Vecsey et al., 2009). These LTP experiments examined SD without prior behavioral training, which more accurately reflects the effects of SD on acquisition of memory. Indeed, it has not previously been shown that SD during consolidation can disrupt LTP. The current study is the first examination of LTP susceptibility to SD after training in a learning task, during consolidation. Our finding that similar SD temporal parameters impair memory and disrupt LTP strengthens the conclusion that sleep is required during this particular time period for memory storage.

We were concerned that prior training would affect LTP induction because a few studies have previously reported alterations in plasticity, in some cases de-potentialization of LTP, after exploration of a novel environment with objects (Kemp & Manahan-Vaughan,

2004; Xu, Anwyl, & Rowan, 1998). We did not observe this de-potentialization of LTP after training in our NSD group. This could be due to reintroduction to the environment and objects over multiple sessions in our paradigm, thereby decreasing the novelty aspect over time. Induction of LTP after introduction to a novel environment has been shown to either enhance LTP induced by weak stimulation or not affect LTP induced by strong stimulation (Dong et al., 2012; Li, Cullen, Anwyl, & Rowan, 2003). We do not see an effect of prior training on cAMP-dependent LTP induced in slices obtained from NSD animals, possibly due to our use of a strong stimulation protocol. Compared to our prior experiences eliciting LTP in slices obtained from non-trained NSD animals, the LTP elicited from prior trained animals did not appear enhanced or reduced (data not shown). Therefore, our LTP findings after training + SD are comparable to our behavioral SD findings because prior exposure to a novel stimulus does not affect resulting LTP in our experiments.

Abnormalities in sleep are accompanied by decreased vigilance, metabolic alterations, and psychiatric disorders (Benca, Obermeyer, Thisted, & Gillin, 1992; Spiegel, Leproult, & Van Cauter, 1999; Stickgold, Whidbee, Schirmer, Patel, & Hobson, 2000). Cognitive deficits are often cited as a result of sleep loss. We have found a narrow critical period during consolidation that is sensitive to the effects of SD. Our finding that the delayed SD post training, rather than the immediate SD post training, impairs both memory and LTP suggests that consolidation processes during the first hour after training are not dependent on sleep. Studies have observed an increase in hippocampal sharp-wave ripples (SPW-R) in the first period of slow wave sleep (Kudrimoti, Barnes, & McNaughton, 1999; Ramadan, Eschenko, & Sara, 2009) critical for memory consolidation and plasticity (Born & Feld, 2012; Chauvette, Seigneur, & Timofeev, 2012; Groszmark, Mizuseki, Pastalkova, Diba, & Buzsáki, 2012). While our findings do not explicitly support this hypothesis about the role of the first period of slow wave sleep, we did not directly examine SPW-R following our behavioral training. Thus, we cannot speak to the importance of this first period SPW-R to sleep-dependent memory consolidation, but future study is warranted.

A potential focus of future research could be exploration of this time window to determine what is being disrupted on a molecular level. Recent research has already begun to examine molecular disruption that occurs with minimal SD, noting disruption in hippocampal cAMP, increased phosphodiesterase 4 (PDE4) enzyme activity in the hippocampus, as well as altered transcription and translation in the hippocampus with only 5 h of SD (Vecsey et al., 2009; Vecsey et al., 2012). However, little is known regarding molecular changes occurring specifically after SD during the consolidation period. Our main findings highlight a time window during which we can begin to explore disruptions in signaling resulting from SD during consolidation, potentially expanding our understanding of molecular mechanisms relevant to both sleep and memory. The continued examination of sleep deprivation and sleep/wake states during consolidation will enable a better understanding of the effects of both sleep and sleep loss on cognition and memory, informing developers of potential novel therapeutic treatments.

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